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Screening for trisomy 21 in Flanders: a 10 years review of 40.490 pregnancies screened by maternal serum

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Abstract

Objective: To evaluate maternal serum screening for trisomy 21 (MSS) in Flanders between 1992 and 2002. *Study design*: Data of a large database on the results of MSS, nuchal translucency (NT) and pregnancy outcome were analysed retrospectively. *Results*: Despite an excellent performance of second trimester MSS at a maternal age ≥35 years (94.4% detection rate (DR) of trisomy 21 at a false positive rate (FPR) of 22.4%), the proportion of patients above 35 years of age in the study population was significantly lower than in the Flemish general pregnant population (5.5% versus 8.9%, $P < 0.001$). In the population screened by MSS and NT, the DR of second trimester MSS at a 5% FPR was 44.4%, which was lower than 66.6% in the population screened by MSS without NT. When nine trisomy 21-affected pregnancies were compared to 3265 normal pregnancies, the mean NT-MoM values were not significantly different (1.16 ± 0.89 versus 1.00 ± 0.46, $P > 0.05$). Both the findings comply to a sequential screening practice where second trimester MSS is only performed after a normal measurement of NT in the first trimester. *Conclusion*: In Flanders, the uptake of second trimester maternal serum screening is low in women aged 35 years or more. Its screening performance decreased after the introduction of sequential screening.

Keywords: Trisomy 21; Second trimester screening; Antenatal invasive procedures; Advanced maternal age; Sequential screening

1. Introduction

There has been a large expansion in trisomy screening strategies over the last 15 years. Screening based on maternal age only has evolved to strategies combining maternal age with maternal serum parameters in the first or second trimester, with and without incorporation of first trimester nuchal translucency measurements (NT) by ultrasound [1,2]. To date screening programs combining first trimester NT with first or second trimester maternal serum parameters and maternal age have been shown to produce the highest sensitivity at a false positive rate of 5% [1–3].

In Flanders, invasive testing is still routinely offered to pregnant women ≥35 years for the antenatal detection of fetal aneuploidy. In trisomy 21 screening programs, second trimester maternal serum parameters were introduced in 1992, followed by NT in 1999 and first trimester serum parameters in 2001. As there are no guidelines on fetal aneuploidy screening in Flanders, a variety of screening methods is used. This study describes the application, performance and evolution of maternal serum screening (MSS) in Flanders over a 10-year-period between 1992 and 2002.

2. Materials and methods

Since 1992 maternal serum samples for second trimester trisomy screening have been analysed by the General Medical Laboratory (AML) in Antwerp, Belgium. These samples were recruited from all geographic regions in Flanders. Immunoradiometric assay was used to measure concentrations of α-foetoprotein (Diagnostic Products Corporation, Los Angeles, USA) and (free) β-human chorionic gonadotropin (BioSource Europe SA, Belgium). Unconjugated estriol levels were measured by radioimmunoassay (Diagnostic Systems Laboratories, Webster, USA).

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Pregnancy-associated plasma protein-A (PAPP-A) was measured by enzyme-linked immunosorbent assay (ELISA-DRG BioSource, Belgium). The cut-off level for a positive screening result was 1:300.

Data on pregnancy and neonatal outcome were obtained from the referring obstetricians after delivery. Once a year, a list was mailed to every clinician for completion of missing data. Non-responding obstetricians were contacted personally to report missing cases of trisomy 21. Accuracy in response rate of trisomy 21-affected pregnancies was evaluated by comparing the prevalence of trisomy 21 in the total screening population to the prevalence in Belgium as registered by Eurocat [4]. The correlation coefficient between the population with completed data and the total screening population was calculated for maternal age distribution and screen positive rates.

The results of second trimester maternal serum screening were analysed retrospectively for different maternal age groups. Data on the distribution of maternal age in the general pregnant population from the Study Centre for Perinatal Epidemiology (SPE) were compared with the maternal age distribution in the study population. Statistical analysis was performed using chi-square analysis.

Since 1999, AML also registered data on first trimester NT (mm) and crown rump length (mm). A total of 2700 measurements were mailed to the Fetal Medicine Foundation for audit according to the FMF-reference range [5]. These data were used to calculate CRL-related multiples of the median (MoM) for every NT-value in our database.

The performance of second trimester MSS combined with NT was evaluated by comparing the detection rate of trisomy 21 (DR) at a fixed false positive rate (FPR) of 5% between MSS only and MSS in combination with NT. Mean NT-MoM values of trisomy 21-affected pregnancies and normal pregnancies were calculated and statistically compared by *t*-test.

Data on the performance of first trimester MMS + NT were limited and will not be discussed further in this paper.

Chromosomal anomalies other than trisomy 21 were not considered in this study.

3. Results

The total number of maternal samples analysed by AML between 1992 and 2002 was 78,365. Complete follow up data were available in 51.7%, leaving a total of 40,490 maternal serum samples eligible for analysis. Table 1 shows the numbers of first and second trimester maternal serum screening tests. A total of 116 trisomy 21-affected pregnancies were reported, indicating a prevalence of 1:675 ratio in the population. Ninety-nine trisomies 21 were found in a total of 67,472 pregnancies screened by MSS only (prevalence 1:682) and nine in 7339 pregnancies screened by MSS with NT (prevalence 1:815). Eight cases were present in the population screened by first trimester MSS and NT.

Table 1
Number and percentage of first and second trimester trisomy screenings performed between 1992 and 2002 with completed data on pregnancy outcome

	n (%)		
	1992–1998	1999–2002	Total
Second trimester MSS	27900 (100)	8482 (67)	36382 (90)
Second trimester MSS + NT	–	3274 (26)	3274 (8)
First trimester MSS + NT	–	834 (7)	834 (2)
Total number	27900 (100)	12590 (100)	40490 (100)

MSS: maternal serum screening; NT: nuchal translucency in the first trimester.

The correlation coefficient between the population with completed data and the total screening population was 0.99, both for maternal age distribution and for screen positive rates.

Table 2 shows the age distribution in the population with complete follow up data of women screened by maternal serum parameters with and without NT, in comparison to the maternal age distribution of the Flemish pregnant population, as registered by the SPE. The proportion of pregnant women ≥35 years was significantly lower in the study population than in the general population (5.5% versus 8.9%, $P < 0.001$). In the total of 78,365 pregnancies screened, 4964 were at maternal age ≥35 years; this was also significantly lower than in the general population (6.3% versus 8.9%, $P < 0.001$).

Table 3 shows the detection rates (DR) and false positive rates (FPR) at a 1:300 risk calculation cut-off for second trimester MSS in different age categories. The overall sensitivity and specificity were 69.7 and 94.5%, respectively. The detection rates and false positive rates were clearly associated with maternal age, i.e. ranging from 50 to 1.5%, in the maternal age category of <20 years to 100 and 32.1% in the maternal age category of >37 years.

Table 2
Comparison of maternal age distribution in the study population vs. the general pregnant population

Maternal age (years)	General population[a]		Study population[b]		*P*-value
	n	%	*n*	%	
<20	13113	2.1	607	1.5	<0.001
20–24	102407	16.4	6559	16.2	ns
25–29	271628	43.5	18990	46.9	<0.001
30–34	181710	29.1	12107	29.9	<0.001
≥35	55575	8.9	2227	5.5	<0.001
Total	624433	100	40490	100	–

[a] Data from the Study Centre for Perinatal Epidemiology (SPE) in Brussels on Flemish deliveries between 1992 and 2002.

[b] Study population data from the database of the General Medical Laboratory (AML) in Antwerp on all first and second trimester screenings with complete data on pregnancy outcome, performed between 1992 and 2002.

Table 3
Detection rates for trisomy 21 and false positive rates of second trimester maternal serum screening as a single test in different maternal age groups

Maternal age (years)	Triple	Trisomy 21		DR (%)	FPR (%)
		+	−		
<20	TT+	2	8		
	TT−	2	539	50.0	1.5
20–24	TT+	1	150		
	TT−	1	5655	50.0	2.6
25–29	TT+	18	606		
	TT−	12	16652	60.0	3.5
30–34	TT+	31	791		
	TT−	14	9926	68.9	7.4
≥35	TT+	17	438		
	TT−	1	1518	94.4	22.4
≥37	TT+	10	213		
	TT−	0	451	100	32.1
Overall	TT+	69	1993		
	TT−	30	34290	69.7	5.5

DR: detection rate; TT+: triple test positive (≥1:300); FPR: false positive rate; TT–: triple test negative (<1:300).

Table 4
Performance of screening by second trimester maternal serum parameters and maternal age with and without inclusion of NT

	Without NT (1999–2002)	With NT (1999–2002)
n tests with data on outcome	36382	3274
n ≥ 35 years (% tests)	1974 (5.4)	259 (7.9)
n trisomy 21	99	9
Detection rate (%)	69/99 (69.6)	5/9 (55.5)
False positive rate (%)	1993/36283 (5.5)	248/3265 (7.6)
[a]Detection rate at FPR = 5%	66/99 (66.6)	4/9 (44.4)

[a] Detection rate at a fixed false positive rate of 5%.

Table 4 shows the performance of second trimester MSS with and without NT. At a fixed FPR of 5% the DR of MSS only was 66.6% and with inclusion of NT was 44.4%. NT did not influence the performance of screening: in the population screened by maternal serum with NT, the DR at a fixed FPR of 5% by the algorithm MSS + age and by the algorithm MSS + age + NT were both 44.4%. The mean NT-MoM values, calculated in the nine pregnancies affected by trisomy 21 and in the 3265 normal pregnancies, were not significantly different (1.16 ± 0.89 versus 1.00 ± 0.46, $P > 0.05$).

4. Discussion

This review of data on trisomy screening provides information on maternal serum screening practices in Flanders between 1992 and 2002. Despite a low overall response rate of 51.7% by Flemish obstetricians contributing to the AML database, the trisomy 21-affected pregnancies were reported adequately: the prevalence of 1:675 in our total screening population is similar to the prevalence of 1:721 registered by Eurocat in Belgium between 1992 and 2001 [4]. The correlation coefficient between the population with complete data and the total screening population was 0.99. We therefore, consider the data of our study group to be representative for the total population screened by MSS.

The proportion of patients ≥35 was significantly lower in our study population than in the general pregnant population (Table 2). We speculate that this effect is the result of primary invasive testing for the indication of maternal age only. In this strategy, maternal serum screening is not performed in pregnancies at maternal age ≥35 years since they are submitted to an invasive procedure. Recently, one of the Belgian centres for Human Genetics reported that 35% of diagnostic invasive procedures in 2001 were performed for maternal age ≥35 years, 28% for positive maternal serum screening and 20% for ultrasonic markers [6]. Advanced maternal age was the first screening parameter used for the identification of a high-risk group for chromosomal anomalies. Wenstrom et al. [7] considered this parameter superior to maternal serum screening for the detection of fetal aneuploidy. Today, it becomes increasingly clear that maternal age alone is a poor parameter to be used in population screening for chromosomal anomalies, both for medical and economical reasons [8,9]. Several reports questioned the practice of routine invasive testing in the older maternal age group and recommended selective invasive testing of only those women who had a positive screening result [10–12]. Our data corroborate this recommendation (Table 2): the sensitivity of second trimester maternal serum screening in our population was much higher at advanced maternal age compared to the younger maternal age group. This has also been reported by others [10,13–15]. We would have performed 1974 amniocenteses by routine invasive testing above 35 years of maternal age (Table 3). Selective invasive testing after prior MSS in this group reduced the number of amniocenteses to 455, missing only one of the 18 pregnancies affected by trisomy 21. This reflects a loss of sensitivity of only 6% for a reduction of 77% of invasive procedures.

A review of screening strategies combining first trimester NT measurements and MSS reports higher detection rates for chromosomal anomalies at a 5% false positive rate, compared to screening by maternal serum parameters or NT only [1]. However, we found a very poor performance of MSS combined with NT (Table 4). The DR of trisomy 21 at a fixed FPR of 5% was only 44.4%, which is much lower than 80–90% reported in literature and also lower than 66.6% by MSS without NT in our population [1]. Adding NT to the MSS algorithm did not alter the DR for trisomy 21 at all. The prevalence of trisomy 21 in the group screened with NT was lower compared to the group screened without NT. These findings can only be explained by changed characteristics of the population screened by maternal serum, after the introduction of NT as a screening parameter in Flanders. As first trimester NT measurement is performed approximately a month before the second trimester maternal serum screen-

ing, it is likely that a proportion of pregnant women were referred for invasive testing when an increased NT value was measured, without having any maternal serum screening test at all. In pregnancies with a normal NT measurement, screening was completed by collecting the second trimester maternal parameters. In this screening strategy, the laboratory would only receive blood samples of the pregnancies with normal NT values. This explains why in our study mean NT-MoM values in the trisomy 21-affected and normal pregnancies were equal, whereas several studies report mean NT-MoM values of 2–2.5 in trisomy 21-affected pregnancies [5,16–18]. This also explains why the DR at a fixed FPR of 5% was only 44.4% in our population screened by MSS with NT whereas this was 66.6% in our population screened by MSS only. Two studies evaluated the impact of screening by first trimester NT measurement on the performance of second trimester MSS [19,20]. Both reported for second trimester MSS a DR of only 50% in the populations screened by first trimester NT, whereas this was much higher when first trimester NT screening was not performed. Our results are very similar to these reports.

The practice of screening in two steps by calculating risks using different parameters at different times, is called sequential screening. At the same DR, the FPR of sequential screening strategies is reported to be at least 30% higher than single step screening, using the same parameters [21]. In order to avoid invasive testing of unaffected pregnancies as much as possible, Flemish obstetricians can be advised to await the screening results of the combined risk calculation of both ultrasound and maternal serum parameters before discussing isolated NT measurement results with their patients. A shift from second trimester screening towards combined first trimester screening may be another solution [22].

In conclusion, our data show a reduction of invasive procedures following second trimester maternal serum screening at maternal age $\geq$35 years, in comparison to primary invasive testing. However, the fraction of the population submitted to this screening method between 1992 and 2002 is lower than expected from the maternal age distribution in the Flemish general pregnant population. After the introduction of NT as a screening parameter in Flanders, the screening performance of the second trimester maternal serum algorithm is apparently lower than before, which appears to be the result of a sequential screening practice. The general introduction of selective invasive testing for positive screening results only at advanced maternal age and of combined single step screening strategies may lead to a further reduction of invasive procedures in normal pregnancies as a result of fetal aneuploidy screening in Flanders.

5. Condensation

The uptake of second trimester maternal serum screening in women aged $\geq$35 years is low. Its screening performance decreased after the introduction of sequential screening.

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